

INSTITUTE REPORT NO. 99



THE MUTAGENIC POTENTIAL OF: 4-nitrophenyl methyl phenyl phosphinate, 4-nitrophenyl diphenyl phosphinate, 4-nitrophenyl dimethyl phosphinate; 4-chlorophenyl methyl phenyl phosphinate,

4-chlorophenyl diphenyl phosphinate

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TOXICOLOGY SERVICES GROUP. **DIVISION OF RESEARCH SUPPORT**

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20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

The Mutagenic Potential of 4-nitrophenyl methyl phenyl phosphinate; 4-nitrophenyl diphenyl phosphinate; 4-nitrophenyl dimethyl phosphinate; 4-chlorophenyl methyl phenyl phosphinate; 4-chlorophenyl diphenyl phosphinate was assessed using the .0000032 Ames Salmonella/Mammalian Microsome Mutagenicity Assay.

Tester Strains TA 98, TA 100, TA 1535, TA 1537, TA 1538 were exposed to doses ranging from 0.01 mg/plate to 3.2×10^{-9} mg/plate for 4-chlorophenyl diphenyl phosphinate and 1 mg/plate to 3.2×10^{-9} mg/plate for all other test compounds. It was determined that none of the tested substances had mutagenic potential

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ABSTRACT

The mutagenic potential of:

4-nitrophenyl methyl phenyl phosphinate	37
4-nitrophenyl diphenyl phosphinate	73/
4-nitrophenyl dimethyl phosphinate	83
4-chlorophenyl methyl phenyl phosphinate	53
4-chlorophenyl diphenyl phosphinate	91

was assessed by the Ames Salmonella/Mammalian Microsome Assay.

Tester strains TA 98, TA 100, TA 1535, TA 1537 and TA 1538 were exposed to doses ranging from 0.01 mg/plate to 3.2×10^{-6} mg/plate for 4 chlorophenyl diphenyl phosphinate and 1 mg/plate to 3.2×10^{-4} mg/plate for all other test compounds. It was determined that none of the tested substances had mutagenic potential.

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PREFACE

SUBSTANCE

AMES	ASSAY	REPORT:	4-nitrophenyl methyl phenyl phosphinate	37
			4-nitrophenyl diphenyl phosphinate	73A
			4-nitrophenyl dimethyl phosphinate	83
			4-chlorophenyl methyl phenyl phosphinate	53
			4-chlorophenyl diphenyl phosphinate	91

TESTING FACILITY: Letterman Army Institute of Research Presidio of San Francisco, CA 94129

SPONSOR: Biomedical Laboratory, Aberdeen Proving Grounds Aberdeen, MD 21005

PROJECT: Toxicity Testing of Phosphinate Compounds - 612772.875

GLP STUDY NUMBER: 80012

STUDY DIRECTOR: LTC John T. Fruin D.V.M., PhD.
CO-PRINCIPAL INVESTIGATORS: SSG Freddica R. Pulliam, B.S.
SP5 Leonard J. Sauers, B.A.

RAW DATA: A copy of the final report, study protocol and retired SOPs will be maintained in the LAIR archives. Test compounds were provided by sonsor. Chemical, analytical, stability, purity, etc. data available from sponsor.

PURPOSE: To determine the mutagenic potential of the above compounds using Ames Salmonella/Mammalian Microsome Mutagenicity Assay. Tester strains TA 98, TA 100, TA 1535, TA 1537, and TA 1538 were used.

Code No.

ACKNOWLEDGMENTS

The authors wish to thank SP5 Lon Kincannon, BA; and SP5 Robert Summers for their assistance in performing the research.

Signatures of Principal Scientists Involved In The Study

we, the undersigned, believe the study described in this report to be scientifically sound and the results and interpretation to be valid. The study was conducted to comply to the best of our ability, with the Good Laboratory Practice Regulations outlined by the Environmental Protection Agency.

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Study Director

DEPARTMENT OF THE ARMY



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8 January 1981

MEMORANDUM FOR RECORD

SUBJECT: Report of GLP Compliance

I hereby certify that in relation to LAIR GLP study 80012 the following inspections were made:

28 October 1980

30 October 1980

18 November 1980

20 November 1980

Routine inspections with no adverse findings are reported quarterly, thus these inspections are also included in the December 1980 report to management and the Study Director.

JOHN L. SZUREK

MAJ, MS

Quality Assurance Officer

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Rationale for using the Ames Assay

The Ames Salmonella/Mammalian Microsome Mutagenicity Test is one of a standard bank of tasts used by our laboratory for the assessment of the mutagenic potential of a test substance. It is a short-term screening assay for the prediction of potential mutagenic agents in mammals. It is inexpensive when compared to in vivo tests, yet is highly predictive and reliable in its ability to detect mutagenic activity and therefore carcinogenic probability (1). It relies on basic genetic principles and allows for the incorporation of a mammalian microsome enzyme system to increase sensitivity through enzymatically altering the test substance into an active metabolite. It has proven highly effective in assessing human risk (1).

Description of Test (Rationale for the schection of strains)

The test was developed by Bruce Ames, Ph.D. from the University of California-Berkeley. The test involves the use of several different genetically altered strains of Salmonella typhimurium, each with a specific mutation in the histidine operon (2). The test substance demonstrates mutagenic potential if it is able to revert the mutation in the bacterial histidine operon back to the wild type and thus reestablish prototrophic growth within the test strain. This reversion also can occur spontaneously due to a random mutational event. If, after adding a test substance, the number of revertants is significantly greater than the spontaneous reversion rate, then the test substance physically altered the locus involved in the operon's mutation and is able to induce point mutations and genetic damage (2).

In order to increase the sensitivity of the test system, two other mutations in the Salmonella are used (2). To insure a higher probability of uptake of test substance, the genome for the lipopolysachride layer (LP) is mutated and allows larger molecules to enter the bacteris. Each strain has another induced mutation which causes loss of excision repair mechanisms. Since many chemicals are not by themselves mutagenic but have to be activated by an enzymatic process, a mammalian microsome system is incorporated. These microsomal enzymes are obtained from livers of rata induced with Aroclor 1254; the enzymes allow for the expression of the metabolites in the mammalian system. This activated and liver microsomal enzyme homogenate is termed 5-9.

Description of Strains (History of the strains used, methods to monitor the integrity of the organisms, and data pertaining to current and historical controls and spontaneous reversion rates)

The test consists of using five different strains of Salmonella typhimurium that are unable to grow in absence of histidine because of a specific mutation in the histidine operon. This histidine requirement is verified by attempting to grow the tester strains on minimal glucose agar (MGA) plates, both with and without histidine. The dependence on this amino acid is shown when growth occurs only in its presence. The plasmids in strains TA 98 and TA 100 contain an ampicillin resistant R factor. Strains deficient in this plasmid demonstrate a zone of growth inhibition around an ampicillin impregnated disc. The alteration of the LP layer allows uptake by the Salmonella of larger molecules. If a crystal violet impregnated disc is placed onto a plate containing any one of the bacterial strains, a zone of growth inhibition will occur because the LP layer is altered. The absence of excision repair mechanisms can be by using ultraviolet (UV) light. determined These mechanisms function primarily by repairing photodimers between pyrimidine bases; exposure of bacteria to UV light will activate the formation of these dimers and cause cell lethality, since excision of these photodimers can not be made. The genetic mutation resulting in UV sensitivity also induces a dependence by the Salmonella to biotin. this vitamin must be added. In order to prove that the bacteria are responsive to the mutation process, positive controls are run with known mutagens. If after exposure to the positive control substance, a larger number of revertants are obtained, then the bacteria are adequately responsive. Sterility controls are performed to determine the presence of contamination. Sterility of the test compound is also confirmed in each first dilution. Verification of the tester strains occurs spontaneously with the running of each assay. value of the spontaneous reversion rate is obtained using the same inoculum of bacteria that is used in the assay (3).

Strains were obtained directly from Dr. Ames, University of California, Berkeley, propagated and then maintained at -80 C in our laboratory. Before any substance was tested, quality controls were run on the bacterial strains to establish the validity of their special features and also to determine the spontaneous reversion rate (2). Records are maintained of all the data, to determine if deviations from the set trends have occurred.

We compared the spontaneous reversion values with our own historical values and those cited by Ames et al (2). Our conclusions are based on the spontaneous reversion rate compared to the experimentally induced rate of mutation. When operating effectively, these strains detect substances that cause base pair

mutations (TA 1535, TA 100) and frameshift mutations (TA 1537, TA 1538 and TA 98) (2).

METHODS (3)

Mark Commence

Rationale for Dosage Levels and Dose Response Tabulations

To insure readable and reliable results, a sublethal concentration of the test substance had to be determined. toxicity level was found by using MGA plates, various trations of the substance, and approximately 10° cells of TA 100 per plate, unless otherwise specified. Top agar containing trace amounts of histidine and biotin were placed on MGA plates. TA 100 is used because it is the most sensitive strain. Strain verification was on the bacteria, along with a determination of the spontaneous reversion rate. After incubation, the growth was observed on the plates. (The auxotrophic Salmonella will rep'icate times and potentially express a mutation. When the biotin supplies are exhausted, only those bacteria that reverted the prototrophic phenotype will continue to reproduce and form macrocolonies; the remainder of the bacteria comprises the background lawn. The minimum toxic level is defined as the lowest serial dilution which decreased macrocolony formation, below that of the revertant rate, and an observable reduction in the density of the background lawn occurs.) A maximum dose of 1 mg/plate is used when no toxicity is observed. The densities were recorded as normal slight, and no growth.

Test Format

After we validated our bacterial strains and determined the optimal dosage of the test substance, we began the Ames Assay. the actual experiment, 0.1ml of the particular strain of Salmonella cells) and the specific dilutions of the test substance were added to 2 ml of molten top agar, which contained trace amounts of histidine and biotin. Since survival is better from cultures which just passed the log phase, the Salmonella strains were used 16 hours (maximum) after initial inoculation into nutrient broth. The dose of the test substance spanned more than a 1000- fold, decreasing from the minimum toxic level by a dilution factor of 5. All the substances were tested with and without S-9 microsome fraction. S-9 mixture which was previously titered at an optimal strength was added to the molten top agar. After all the ingredients were added, the top agar was vortexed, then overlayered on minimum glucose agar plates. These plates contained 2% glucose and Vogel Bonner Concentrate (4). The water used in this medium and a'l reagents came from a polymetric system. Plates were incubated, upside down in the dark at 37 C for 48 hours. Plates were prepared in triplicate and the average revertant counts were recorded. The corresponding number of revertants obtained was compared to the number of spontaneous

revertants; the conclusions were recorded statistically. A correlated dose response is considered necessary to declare a substance as a mutagen. Commoner (5), in his report, "Reliablilty of Bacterial Mutagenesis Techniques to Distinguish Carcinogenic and Non-Carcinogenic Chemical," and McCann et al (1) in their paper, "Detection of Carcinogens as Mutagen: Assay of over 300 Chemicals," have concurred on the test's ability to detect mutagenic potential.

Statistical Analysis

Quantitative evaluation was ascertained by two independent methods. Ames et al (2) assumed that a compound which caused twice the spontaneous reversion rate is mutagenic. Commoner (5), developed the MUTAR Ratio, which is stated in the following equation:

$$MUTAR = (E - C)/C_{AV}$$

Here, C is the number of spontaneous revertant colonies on control plates obtained on the same day and with the same treatment and strains. E is the number of revertants in response to the compound; C_{AV} is the number of spontaneous revertants on control plates calculated from historical records. The explanation of the results of this equation can be determined by the method of Commoner (5). This variation determines the probability of correctly classifying substances as carcinogens on the basis of their mutagenic activity. The E values were recorded by strain, with and without S-9. Values for C and C_{AV} were recorded separately.

We used the formula and logged all values for our permanent records.

RESULTS AND DISCUSSION

Throughout this report, all the test substances will be referred to by their respective code numbers:

Substance	Code No.
4-nitrophenyl methyl phenyl phosphinate	e 37
4-nitrophenyl diphenyl phosphinate	73A
4-nitrophenyl dimethyl phosphinate	دَ8
4-chlorophenyl methyl phenyl phosphinat	te 53
4-chlorophenyl diphenyl phosphinate	91

A series of assays was run to conclusively determine the mutagenic potential of the five substances. Data from tests that were determined to be invalid due to medium preparation errors, inadequate inoculum or control failures are not reported but are retained in the LAIR archives. On 4 Nov 80, the Ames Test was performed on 73A and 37. Due to an error in medium preparation, no

growth was present after the 48-hour incubation. This assay was repeated on 12 Nov 80. On 18 Nov 80, substances 53, 83 and 91 were tested. Throughout the assays of 12 and 18 Nov, we observed uneven lawns on plates containing test strain TA 1537. We suspected that the TA 1537 inoculum was insufficient; therefore, all five chemicals were retested on 2 Dec 80, using an inoculum of TA 1537 prepared from parent culture stock. A plating error resulted in a lack of growth on the positive control plates. The test was done again on 9 Dec 80. The spontaneous reversion level was below our historical data for nonactivated TA 98 and nonactivated TA 1538 from the 18 Nov 80 assay. The experiment was repeated on 16 Dec 80 with only TA 98 and TA 1538.

Strain verification and sterility controls were normal for all assays reported (Tables 1A - 1E). The assay of 12 Nov 80 showed a spontaneous reversion rate below that suggested by Ames et al (2) on both activated and nonactivated TA 98, TA 100, and TA 1538 also for activated TA 1535 (Table 1A). On 18 Nov 80, all the spontaneous reversion rates for the nonactivated strains were below that suggested range along with activated TA 1535 and TA 1538. Nonactivated TA 98 and TA 1538 (Table 1B) were significantly below our historical data values. The spontaneous reversion rate was low for TA 98 nonactivated on 16 Dec 80 (Table 1E). Spontaneous reversion values below that suggested by Ames et al (2) are indicative of high quality water, materials, techniques, etc. Counts higher than those suggested by Ames et al (2) are indicators of serious performance

The effects of the positive control chemicals are reported in Tables 2A - 2D. Positive control values below that expected were observed for TA 98, TA 1537 and TA 1538 to dimethyl-benzanthracene (DMBA) on 12 Nov 80 (Table 2A). On 18 Nov 80, the same results were seen for TA 98, TA 100, TA 1537 and TA 1538 (Table 2B). Below par value were also evident on 9 Dec 80 for TA 1537 to DMBA. The same was true on 16 Dec 80 for TA 98 and TA 1537 (Tabel 2D). DMBA functions as a frameshift mutagen and is used to determine if strains TA 98, TA TA 1537 and TA 1538 are functioning properly. Although the strains did not respond to DMBA, they did respond to aminofluorene (AF) and benzo()pyrene (BP), both of which are also frameshift mutagens. In all instances when n-methyl-n nitro-N-nitrosoquanidine (MNNG) was the positive conrol, test strains responded as anticipated.

The Minimum Toxicity Level Determination Assay was performed on $28\ \, 0ct\ \, 80.$ Our quality control showed that we had incurred experimental contamination on the test plates (Table 3). By observing the condition of the background lawn, the optimal sublethal dose was determined, even though extraneous growth was present. Sparse or no growth of the background lawn signified toxicity. The optimal sublethal dose was chosen at a point where a lawn having normal growth became evident (Table 4A-4E).

The data for the mutagenic potential are reported in Tables 5A - 5J. Data for test compound 37 were collected on 12 Nov 80 and 11 Dec 80. On 12 Nov 80 (Table 5A), two isolated incidences of a more than doubling of the spontaneous reversion rate occurred: activated TA 1535 at the 0.0016 mg/plate dose and activated TA 1537 at the 0.04 mg/plate level. The assay of strain TA 1537 was performed again on test substance 37 on 11 Dec 80 (Table 5G). No mutagenic activity was demonstrated. It is concluded that the response of activated TA 1537 on 12 Nov 80 for the 0.04 mg/plate dose was unexplainable and probably due to experimental error since the results could not be reproduced. The activity found in TA 1535 was disregarded due to the lack of correlation with dose response.

The data for test substance 73A were obtained on 12 Nov 80 (Table 5B) and 11 Dec 80 (Table 5G). In all occurences, no evidence of mutagenic activity was found.

Compound 83 was tested on 18 Nov 80 (Table 5C), 11 Dec 80 (Table 5G) and 16 Dec 80 (Table 5H). On 18 Nov 80, all TA 98 dose levels showed doubling or greater of the spontaneous reversion rate. This was also true for nonactivated TA 1538 at the 0.04 mg/plate dose level through the 0.00032 mg/plate dose. The spontaneous reversion rate for these nonactivated strains was below that suggested by Ames et al (2) as indicative of mutagenicity. Test substance 83 was assayed using only strains TA 98 and TA 1538 on 16 Dec 80. No mutagenic activity was presented. It was concluded that the 18 Nov 80, suggestion of mutagenic activity was due to the spontaneous reversion rate for TA 98 and TA 1538 which was far below the historical average. The MUTAR values were also insignificant.

Test substance 53 was tested on 18 Nov 80 (Table 5D), 11 Dec 80 (Table 5G), and 16 Dec 80 (Table 5I). On 18 Nov 80, a greater than twice the spontaneous reversion rate was observed for all dose levels containing nonactivated TA 98 and nonactivated TA 1538. The same occurred for nonactivated TA 1535 at the 0.008 and 0.00032 mg/plate doses. Nonactivated TA 1537 showed possible mutagenic activity at the 0.008 and 0.0016 mg/plate dose levels. On 11 Dec 80, the TA 1537 assay was repeated; no evidence of mutagenic activity was present. It was concluded that the mutagencity initially presented with TA 98, TA 1537 and TA 1538 was due to low spontaneous reversion rates. The activity found in TA 1535 was disregarded due to the low spontaneous reversion rate and the lack of correlation to dose response.

Test substance 91 was assayed on 18 Nov 80 (Table 5E), 11 Dec 80 (Table 5G) and 16 Dec 80 (Table 5J). In the assay of 18 Nov 30, a doubling or greater spontaneous reversion rate was seen for nonactivated TA 1558 and nonactivated TA 98 for all dose levels. The spontaneous reversion values were low for both of these nonactivated strains. When the assay was repeated on 11 Dec 80, no mutagenic

activity was seen. It was concluded that the initial observation of mutagencity was due the low spontaneous reversion values. All calculated MUTAR values were below the 1.5 threshold value necessary to declare a substance as a mutagen (Tables 6A-6M).

CONCLUSION

To declare that a substance is a mutagen through the Ames Test, two criteria must be met: a more than doubling of the spontaneous reversion rate and an obvious dose response. Since only a few scattered incidences of twice the spontaneous reversion rate were observed, it was concluded that compounds 37, 73A, 83, 53 and 91 are not mutagenic.

RECOMMENDATION

We recommend that organo-phosphinate compounds 37, 73A, 83, 53, and 91 be tested using other toxicological testing systems if efficacy tests show those chemicals to be promising antidotes.

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APPENDIX (Continued)

Table 1-A

QUALITY CONTROL OF TESTER STRAINS WORKSHEET
Salmonella/Microsome Assay

					7: (:					
Strain No.	Histidi Require			cillin stance		uvr-B Delet		rfa Cr Violet	ystal (d)	Sterility Control (e)
TA 98	+			+		+		16.2	7 mm	NG
TA 100	+			+		_+		16.6	5 mm	NG
TA 1535	+			NA		+		20.3	5 mm	NG
TA 1537	+	,		26.05	mm	+		18.3	4 mm	NG
TA 1538	+			NA		+		20.3	O mm	NG
WT	grow	th		NA		NA		NA	I	NA
			Δι ττν	CONTR	201 (4				i	
His-Bio mix	Initi	11: _ ^{NT} _		End:	NT	<i>=</i> /	T	act Comm	ound 1	+ (73A)
Top Agar		11:		End:	+			est Comp		
i		il: <u>+</u>		End:			, T	est Comp	ound 3:	NA NA
Diluent:	+			Nutrie	nt Br	oth:_ 	т	est Cpmp	ound 4:	tiA
MGA Plate w/ I	pacteria:	+		MGA P1	ate:_	+	т	est Comp	ound 5:	NA
(a) + = no gru - = zone of in side of plate growth (growth tlA = not appli	nhibition ; (d) + h indicat	of appr zone of tes conta	oxima of inh uminat	tely labition;	6mm; on app NT=no	(c) + roximat	≈ no tely ì ed; NG	growth d Amm diam	n irrac meter: (diated (e) + = no
Strain (1)	Avg I	Range	No	S-9		Avg		S-9		Avg
TA 98	40	30-50	25	24	18	22	32	24	23	26
TA 100	160 12	20-200	102	121	122	115	112	102	123	112
TA 1535	20	10-35	14	16	8	13	6	3	5_	5
TA 1537	7	3-15	14	11	.8	11	15	4	10	_10
TA 1538	25	15-35	6	14	11	10	13	13	15_]4

Ames, B.N., J. McCann and E. Yamasaki. Mucat. Res. 31:347

Test Inoculated By:	Summers, Sauers, Pulliam, Kinca	nggile.	12 Nov 80	_
Test Read By: Pullia	m	Date:	14 Nov 80	

Table-1-B

QUALITY CONTROL OF TESTER STRAINS WORKSHEET Salmonella/Microsome Assay

		<u> </u>							
Strain No.	Histidine (a) Requirements		cillin stance		uvr-B Delet		rfa Cr Violet		Sterility Control (e)
TA 98	+		+		+		15.33	men	7G
TA 100	÷		+				17.22		NG
TA 1535									NG
TA 1537	+ .		2.43		+		17.44	mm	NG
TA 1538	+		NΑ		+		20.0m	m	11G
WT	Growth		HA.		NA.		NA_	<u> </u>	NT.
QUALITY CONTROL (e)									
His-Bio mix	Initial:	+	End:	+		Te	st Comp	ound 1:	83- NG
Top Agar	Initial:	+	End:	+		Te	st Comp	ound 2:	91- NG
S - 9	Initial:	+	End:	+		. Te	st Comp	ound 3:	53- NG
Diluent:	+		Nutrie	ent Br	oth: <u>+</u>	Te	st Cp mp	ound 4	NA
MGA Plate w/	bacteria: <u>Growt</u>	h	MGA P1	late:_	+	Te	st Comp	ound 5	: <u>\\</u> A
- = zone of i	owth (requires inhibition of ap; (d) + = zone h indicates contable.	proxima of inh taminat	itely initional initionali	l6mm; on app NT=no	(c) + roximat	= no g ely 14 ed; NG=	prowth c Amm diam	n irra eter:	d1ated (e) + = no
Strain (1)	Avg Range	No	5-9		Avg		S-9		Avg
TA 98	40 30-50	4	0	3	2	29	41_	38	36
TA 100	160 120-200	78	63	88	76	133	140	118	130
TA 1535	20 10-35	8	6	2	5	12	2	3	6
TA 1537	73-15	*3	0	7_	3	<u> </u>	1_1_	8	5
TA 1538	25 15- <u>35</u>	1	4_	<u> 1</u>	1 2	6	1 13	<u> </u> 13	<u> </u>
). McCann and E.								
Test Inocula	ted By: <u>Sauers</u> ,	Summe	rs, Pu	<u>Neill</u>					
Test Read By	· Pulliam					Date:	20 №	ov 80	

* Sparse lawn

Table-1-C

QUALITY CONTROL OF TESTER STRAINS WORKSHEET Salmonella/Microsome Assay

										·
Strain No.		dine (a) rements		cillin stance		uvr-b Delet	(c) ion	rfa C Viole	rystal t (d)	Sterility Control (e)
TA 98		NA		NA NA			4	<u>N</u> A		NA.
TA 100		NA		NA		N/	A	NA		NA
TA 1535	<u> </u>	+		MA		N/	4	<u>NA</u>		NA .
TA 1537		+		18mm		<u>+</u>		20m	m	4G
TA 1538		NA		MA		!#/	1	NA.		NA NA
WT	Gro	owth		NA		٧/	1	<u>N</u> A		NA NA
		QL	JALITY	CONTR	<u>OL</u> (e)				
His-Bio mix	Init	ial: <u>+</u>		End:	+		T	est Com	pound 1	: <u>!IG</u>
Top Agar	Init	ial: <u>+</u>		End:	+		T	est Com	pound 2	: NA
S - 9	Init	ial: <u>+</u>		End:	+		٦.	est Com	pound 3	3: <u>NA</u>
Diluent:	TN			Nutrie	nt Br	oth: <u>+</u>	1	est Cpm	pound 4	: NA
MGA Plate w/	bacteri	a:+		MGA P1	ate:_	+	1	est Com	pound 5	5: <u>NA</u>
(a) + = no jr - = zone uf i side of plate growth (growt NA=not applic	; (d) h indic	+ = zone (ates conta	of int uminat	nibitio	n app NT=no	roxima: t test	tely l ed; NG	4mm dia	meter;	(e) + = no
Strain (1)	Avg	Range	No	S-9		Avg	<u> </u>	S-9		Avg
TA 98	40	30-50	T			NA		1		l na
TA 100	160	120-200				NA :				1A
TA 1535	20	10-35	T			NA				NA .
TA 1537	7	3-15	4	4	3 .	4	7	8	2	6
TA 1538	25	15-35				NA			<u> </u>	l _{NA}
Ames, B.N., J). McCai	nn and E.	· Ya.mas	aki. I	Autat.	Res.	31:34	7		

Test	Inoculated	By: Sauers, Summers, Pulliam	Date:	2 Dec 80
Test	Read By: _	Fullian	Date:	4 Dec 30

^{*} Tested when batch first made, see data dated 18 Nov 80

Table-1-D

QUALITY CONTROL OF TESTER STRAINS WORKSHEET Salmonella/Microsome Assay

Strain No.	Histidine (a) Requirements		icillin istance		uvr-l Delei	(c) tion		rystal t (d)	Sterility , Control (2)	
TA 98	<u>NA</u>		NA		NA NA		N/		NA.	
TA 100	NA NA		NA		NA		N/	1	NA NA	
TA 1535	NA NA		NA		ŅΑ		N/	١	NA .	
TA 1537	+	<u> </u>	1 GMM		+		1.7r	nm	NG	
TA 1538	NA NA		NA		NA		N/	1	NA NA	
WT	NA NA	<u> </u>	NA		NA		N/		N3	
QUALITY CONTROL (e)										
His-Bio mix Initial: + End: + Test Compound 1: NG										
Top Agar	Initial: <u>1 co</u>	lony	End:	+	_	T	est Con	pound 2	: NG	
S - 9	Initial: +		End:	+		ر ٦	est Con	pound 3	: <u>NG</u>	
Diluent:	Diluent: + Nutrient Broth: + Test Cpmpound 4: NG									
MGA Plate w/ b	cacteria: <u>(WT) g</u>	<u>row</u> th	MGA P1	ate:_	+	T	est Com	pound 5	: <u>NG</u>	
- = zone of ir	with (requires he hibition of app. (d) + = zone of indicates contable.	roxima of inl amina	ately 10	6mm; n appi NT=no	(c) + roximat t teste	= no tely l ed; NG	growth	on irra	diated	
Strain /	Avg Range] No	S-9		Avg	 	S- 9		J Avg	
(1)		-	, , , , , , , , , , , , , , , , , , ,		ļ			1	-	
TA 98	40 30-50				NA :		 	 	NA I	
TA 100	60 120-200		 		NA	<u> </u>		 	NA	
TA 1535	20 10-35	-	<u> </u>		NA .		<u> </u>	ļ	NA	
TA 1537	7 3-15	9_	8	5	7	_9_	8	6	3	
TA 1538	TA 1538 25 15-35 NA NA									
Ames, B.N., J. McCann and E. Yamasaki. Mutat. Res. 31:347										
Test Inoculated By: Pulliam, Sauers Date: 9 Dec 80										
Test Read By:	Pulliam					Date	: 11 0	ec 80	and the same and the same	

Table-1-E

QUALITY CONTROL OF TESTER STRAINS WORKSHEET
Salmonella/Microsome Assay

Strain No.	Histidine (&) Requirements		cillin (istance	(p)	uvr-8 Delet	(c) ion	rfa Cr Violet	ystal (d)	Sterility Control (e)		
TA 98	+		+		+		17.43	lmm	NG		
TA 100	+		NA		t_		<u>IIA</u>		NA		
TA 1535	NA		NA		NA.		NV		NA		
TA 1537	NA ·		NA	_	NA		NA		AK		
TA 1538	+		23.45		+		17.21	mm	NG		
<u>kt</u>	NA NA		NA		Grow	.h	NA.		AI:		
	QU	ALITY	CONTROL	_ (e	e)						
His-Bio mix Initial: + End: + Test Compound 1: + (53)											
Top Agar Initial: + End: + Test Compound 2: + (91)											
S - 9											
Diluent:	Diluent:										
MGA Plate w/ b	oacteria: +		MGA Plat	te:	+	T	est Comp	ound 5	: <u>*IA</u>		
(a) + = no gro - = zone of in side of plate growth (growth NA=not applica	with (requires hinibition of appr (d) + = zone of indicates conta ble. Spo	oxima f ini minat	ine for quately 16million tion); Niceous Reve	mm; appi [≈not	(c) + roximat teste	= no cely l ed; NG	= no zon growth o 4mm diam =no grow	e of 11 n irradeter; th; WT:	enthition, diated (e) + = no wild type		
Strain /	Avg Range	No	S-9		Avg		S-9		Avg		
TA 98	40 30-50	23	24 2	28	25	25	34	32	30		
TA '00' AT	160 120-200				NA				<u> </u>		
TA 1535	20 10-35				'VA				NA		
TA 1537	7 3-15	<u> </u>			NA.				NA		
TA 1538	25 15-35	111	14	19	15	20	15_	17	13		
Ames, B.N., J. McCann and E. Yamasakı. Mutat. Res. 31:347											
Test Inoculated By: Pulliam, Summers, Sauers Date: 16 Dec 80											
Test Read By:	Sauers, Sun	mers				Date	12	<u>Dec 80</u>	and and and		

TABLE 2 A

POSITIVE CONTROL REVERTANT RATE

Date	Strain	Spontar	ieous Rev	AF	MNNG	BP	DIIBA	Re-	Init
Date	Strain	S-9	No S-9	s- 9	No S-9	S-9	S-9	sponse (a)	11110
14 Nov	TA 98	26	22	1453	NA	137	48		
н	TA 100	112	115	728	5109	370	227	+	
"	TA 1535	5	13	NA	16104	NA	NA	1 .	
"	TA 1537	10	11	NA	NA	38	18	-	
"	TA 1538	14	10	1619	NA	77	18	<u> </u>	
						<u> </u>			
							<u> </u>	<u> </u>	
		1							
L					<u> </u>				
	<u> </u>	<u> </u>						<u> </u>	
		<u> </u>							
					<u> . </u>				
				<u> </u>			<u> </u>		
							1		

(a) + = expected result, - = unexpected result (see discipling note) TA 98, TA 1537, and TA 1538 showed an unexpected low response to DMBA.

TABLE 2-B
POSITIVE CONTROL REVERTANT RATE

Date	Stz · in	Spontar	eous Rev	AF	MNNG	BP	DEBA	Re-	Init
Date	201	2-9	No S-9	s - 9	No 5-9	S-9	S-9	sponse (a)	
18 Nov	TA 98	36	2	851	NA	83	43		
#	TA 100	130	76	475	1955	161	1117_		<u> </u>
11	TA 1535	6	5	NA	1392	NA	NA	+	
11	TA 1537	5	3	NA	NA.	28	9	<u> </u>	
#	TA 1538	11	2	980	NA_	26	18	<u> </u>	<u> </u>
	1	<u> </u>							<u> </u>
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······································	<u> </u>	 	+		+	i 	 	-	

(a) += expected result, -= unexpected result (see discipline note)
TA 93, TA100, TA 1537, and TA 1538 showed an unexpected low response
to DMBA.

TABLE 2-C
POSITIVE CONTROL REVERTANT RATE

Date	Strain	Spontan	eous Rev	AF	MNNG	BP	DUBA	Re-	Init
Mte	Strain	S-9	No S-9	s-9	No S-9	S-9	S-9	sponse	
4 Dec	TA 1537	6	4	NG	NA	#IG	NG	-	
9 Dec	TA 1537	8	7	14	NA	26	13	-	
 	 								
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 		 					<u> </u>		
 	<u> </u>	<u> </u>							-
} -							-		
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 		-						-	-
		 			 		 	 	
-		<u> </u>	 	 -	<u> </u>		 	 	
-		 	 		 	<u> </u>	 	 	
		1	1		<u> </u>	<u> </u>		1	1

(a) + = expected result, - = unexpected result (see discipline note)

Plating error caused lack of growth in assay of 4 Dec. TA 1537 showed an unexpected low response to DHBA in assay of 9 Dec.

TABLE 2-D
POSITIVE CONTROL REVERTANT RATE

	Strain	Spontan	eous Rev	AF	MNNG	BP	DUBA	Re-	Init
Date	Strain	s-9	No S-9	S-9	No S-9	S- 9	s- 9	sponse (a)	11.10
16 Dec	TA 98	30	25	1055	NA.	59	26		
11	TA 1538	18	15	984	NA.	50	28		
	<u> </u>								
				<u></u>					
							 		
	<u> </u>	-			 		+	 -	<u> </u>
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<u> </u>					<u> </u>		<u> </u>	<u> </u>	
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<u> </u>		-				 	 	-	<u> </u>
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	 	 	-	 	 		 	-	1
		 	 	 	 		+	1	
 	 	 	<u> </u>		 			1	
						<u> </u>	1		

(a) - = expected result, - = unexpected result (see discipline note)

TA 98 and TA 1538 showed an unexpected low response to DMBA. TA 98 showed an unexpected low response to BP.

Table-3

STRAIN VERIFICATION FOR TOXICITY LEVEL DETERMINATION Salmonella/Microsome Assay

Strain No.	Histidine (a) Requirements	Ampicillian (b) Resistance	uvr=B (c) Deletion	rfa Crystal Violet (d)	Sterility Control (e						
TA 100	+	+	+	15mm	NG						
TA 1537	NT	24 mm	NT	NT	NG						
WT	Growth	NT	Growth	NT	NT						
Diluent	NT	NT	NT	NT	NG						
Test Compound (s	5)				3						
#1	NT	NT	NT	ти	nt						
#2	NT	NT	NT	NT	7						
#3	NT	NT	NT	NT	nue.						
#4	•	NT	NT	NT	1						
# 5	NT	NT	NT	NT	6.						
(a) + = no growth (requires histidine for growth); (b) + = no zone of inhibition, - = zone of inhibition of approximately 16mm; (c) + = no growth on irratiated side of plate; (d) + = zone of inhibition approximately 14mm diameter; (e) + = no growth (growth indicates contamination); NT=not tested; WT= wild type. Spontaneous Revertants											
Strain	Average Ra	inge			Average						
			 	un aton							

Test Inoculate	d By: <u>Sauers, Summers, Kellner</u>	Date: <u>28 Oct 90</u>
Test Read By:	Pulliam	Date: 31 Oct 90

Table 4-A TOXICITY LEVEL DETERMINATION Salmonella/Microsome Assay

Substance assayeu:	·				
(3)	(4)		(5)	
Date:	Perfor	rmed by: <u>s</u> a	wers. Kinc	nnon, Pulliam	Summers
Substance dissolved	in: (1) <u>DMS</u>	(2)		(3)	
(4)(5)			l estimatio ent Agar Pl TA 100 nt Plate Co	ST = s1 NL = no	d lawn on growth ight growth rmal growth
Test Compound Concentration	Plate #1			Average	Background Lawn
1.0 mg/plate					NL
0.1 mg/plate	-	XC			NL
0.01 mg/plate			ROX OF THE PARTY O		NI
0.001 mg/plate			Arrest.		и
0.000.1 mg/plate	<u> </u>				NL NL
0.000.01 mg/plate	 				NI
0.000,001 mg/plate					NI.
0.000,000,1_mg/plate					NI.
			-		
	ļ				
	i	1			

Table 4-B

TOXICITY LEVEL DETERMINATION Salmonella/Microsome Assay

Substance assayed:	(1) <u>Code #</u>	73	(2)					
(3)	(4)		(5)				
Date:	Perfo	med by: <u>Sauers, Kincannon, Pulliam, Summers</u>						
Substance dissolved	in: (1) <u>DMS</u>	<u>o</u> (2)		(3)				
(4)(5)		Visua Nutri	l estimatio ent Agar Pl TA 100	n of backgroun ates: NG = no ST = s1 NL = no	d lawn on growth ight growth rmal growth			
Test Compound Concentration	Plate #1	Reverta	nt Plate Co		Background Lawn			
1.0 mg/plate					ST			
0-1 mg/plate					NL			
0.01_mg/plate		\rightarrow			NL			
u.001_mg/plate			Lever		NI.			
0.000.1_mg/plate			Son Con Rect		- NT			
0.000.01 mg/plate	ļ				NL			
0.000.001 mg/plate					NI			
0.000,000,1 mg/place					NL			
	<u> </u>				<u> </u>			

Table 4-C
TOXICITY LEVEL DETERMINATION
Salmonella/Microsome Assay

Substance assayed:	(1) <u>Code #</u>	83	(2)		
(3)	(4)		(5)	
Date: <u>28 Oct 80</u>	Perfor	med by: <u>sa</u>	uers. Kinca	innon, Pullian,	Summers
Substance dissolved	in: (1) <u>DMS</u>	<u>o</u> (2)		(3)	
(4)(5)	***************************************	Visua Nutri	ent Agar Pl TA 100	NL = nor	lawn on growth ght growth mal growth
Test Compound			nt Plate Co		Background
Concentration	Plate #1	Plate #2	Plate #3	Average	Lawn
1.0 mg/plate					NL
0.1 mg/plate					NL NL
0.01 mg/place		S			NI.
0.001 mg/plate		,,,	وكو		NI.
0.000.1 mg/plate			- Grand		NI.
0.000.01 mg/plate	ļ		X port by the XX	u .	NL.
0.000,001 mg/plate	ļ				NI.
0.000,000,1 mg/plate					NL

* Table 4-D TOXICITY LEVEL DETERMINATION Salmonella/Microsome Assay

Substance assayed:	(I) Code	#53	(2) .		
(3)	(4)		(5)	~
Date:	Perfor	med by: <u>s</u> a	uers. Kinca	nnon, Pulliam	. Summers
Substance dissolved	in: (1) <u>DMS</u>	<u>o(2)</u>		(3)	
(4)(5)	-		l estimatio ent Agar Pl FA 100	ST = sl	nd lawn on growth light growth ormal growth
Test Compound Concentration	Plate #1		nt Plate Co Plate #3	unt Average	Background Lawn
O mg/plate					NL NL
.1 mg/plate					NT NT
.01 mg/plate		74	X R. A. A. A. A.		NL NL
.000.1 mg/plate			* Parker	K	NI.
.000.01 mg/plate				2	NI.
.000,001 mg/plate .000,000,1 mg/place	1				NI.
		!			
		.i		L	

Table 4-E TOXICITY LEVEL DETERMINATION Salmonella/Microsome Assay

Substance assayed:	(i) Code	#91	(2)		
(3)	(4)		(5)	
Date:28_Oct_80	Perfor	rmed by: <u>Sa</u>	wers. Kinc	innon, Pullia	m. Summers
Substance dissolved				(3)	
(4)(5)	***************************************	Visua Nutri	l estimatio ent Agar Pl TA 100	n of backgro ates: NG = : ST = NL =	und lawn on no growth slight growth normal growth
			nt Plate Co	unt	
Test Compound Concentration	Plate #1			Average	Background Lawn
1.0 mg/plate					NG
0.1 mg/plate					st
0.01 mg/plate		S			NL
0.001 mg/plate		7	6		NL
0.000.1 ing/place	<u> </u>		X and XX		NL
0.000,01 mg/plate	<u> </u>			2	NL
0.000,001 mg/plate	<u> </u>				NI.
0.000,000,1 mg/plate	ļ				NI.
	<u> </u>				

Table-5-A
SALMONFLLA/MICROSOME ASSAY WORKSHEET
(POSITIVE CONTROLS/TEST COMPOUND)

	Substance Assa	yed: (1)	Code	#37	(2)				
	(3)		(4)			(5) _	÷ .,			
	Date: 12 Nov 80 Performed By: Sauers, Pulliam, Kincar									Summers	
	Substance diss	o1 ved	in: (1) <u>DMSO</u> (2)								
	(3)		(4)(5)								
		1	•	# R	levertan	t/Plate	_				
Sub	Conc	98	98A	100	100A	1535	1535A	1537	1537A	1538	1538A
37	1.0 mg/p1	12	13	60	66	6	5	6	7	5	7
37	0.2 mg/p1	12	19	83	92	10 .	4	5	7	10	21
37	0.04 mg/pl	14	28	89	97	6	7	9	110	10	25
37	0.008 mg/p1	21	23	75	88	11	6	8	8	9	17
37	0.0016 mg/p1	18	22	84	77	10	12	7	12	12	14
37	0.00032 mg/pl	18	19	112	89	12	6	6	7	5	13
	Spon. Rev.	22	26	115	112	13	5	11	10	10	14
						·					

Table-5-B

SALMONELL A/MICROSOME ASSAY WORKSHEET (POSITIVE CONTROLS/TEST COMPOUND)

	Substance Assa	yed:	(1)	Code	#73A	((2)				
	(3)	(4)			(5)					
	Date: 12 Nov	80		_ Perf	ormed B	ly: <u>Kinc</u>	y: <u>Kincannon, Summers, Sauers, Pulli</u> am				
	Substance dissolved in: (1) <u>DMSO</u>					(2)					
	(3)		(4)			(5)				
		•	•	# R	evertar	t/Plate	<u>_</u>				
Sub	Conc	98	98A	100	100A	1535	1535A	1537	1537A	1538	15384
73A	1.0 mg/p1	9	14	47	54	9	55	44	2	8	15
73A	0.2 mg/p1	14	21	63	68	8	7	_5	_6_	_10_	14_
73A	0.04 mg/pl	16	21	80	90	10_		8	6	8	22
73A	0.008 mg/pl	14	16	65	90	7	8	10	6	_6_	12_
73A	0.0016 mg/pl	12	24	74	95	7	8		9	10	14_
73A	0.00032 mg/p1	13	15	76	80	5	9	5	1_2	8	14_
			ļ	ļ							
ļ	Spon. Rev.	22	26	115_	112	13	5		10	10	14
	-	<u> </u>	 						ļ		·····
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Table-5-C SALMONELLA/MICROSOME ASSAY WORKSHEET (POSITIVE CONTROLS/TEST COMPOUND)

	Substance Assa;	yea:	(1)	Jue #0.	,	١ ــــــ ١	.41				
	(3)		(4)			(5) _	 			
	Date: 18 Nov 8	30		Perf	ormed B	y: <u>Kin</u>	cannon, S	ummers,	Sauers,	<u>Pull</u> ia	m
	Substance diss	olved	in: (1) <u>D</u>	150	······································	(2)				
	(3)		(4)			(5)		<u> </u>		
		1	٠	# R	evertan	t/Plate	<u>.</u>				
Sub	Conc	98	98A	100	100A	1535	1535A	1537	1537A	1538	1538A
83	1.0 mg/p1	11	10	47	40	4	6	1	1	4	7
83	0.2 mg/pl	19	22	81	71	9	7	3	4	3	12
83	0.04 mg/pl	17	19	84	96	5	7	5	3	6	12
83	0.008 mg/pl	19	22	85	84	6	6	3	3	7	15
83	0.0016 mg/p1	20	17	66	60	6	4	1	4	13	7
83	0.00032 mg/p1	12	15	51	83	6	7	3	2	6	11
								•			
	Spon. rev.	2	36	76	130	5	6	3	5	2	11
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Table-5-D SALMONELLA/MICROSOME ASSAY WORKSHEET (POSITIVE CONTROLS/TEST COMPOUND)

	Substance Assa	yed: ((1) <u>Co</u>	de #53		(2)				
	(3)		(4	·)			1535 1535A 1537 1537A 1538 1 6 4 3 2 14 8 7 4 3 9 9 9 2 5 5 12 7 6 5 11 6 9 9 6 6 9 13 8 3 6 9				
	Substance diss	olved	in: (1)	DHSO		(2)				
	(3)		((4)	, 		(5)		<u></u>		
		•	٠	# 18	evertar	t/Plate					
Sub	Conc	98	98A	100	100A	1535	1535A	1537	1537A	1538	1538A
53	1.0 mg/p1	17	25	66	74	6	44	3	2	14	13
53	0.2 mg.pl	15	20	89	86	88	1	4	3		
53	0.04 mg/pl	17	18	75	85	9	9	2	5	5	1L_
53	0.008mg/p1	17	23	84	93	12	7	6	5	_11_	16
53	0.0016 mg/p1	16	25	87	82	6	9	9	6	6	16
53	0.00032 mg/p1	13	25	70	90	13	8	3	6	9	u_
					<u> </u>						
~~~~	Spon. Rev.	2	36	76	130	5	6	3	5	2	
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Table-5-E
SALMONELLA/MICROSOME ASSAY WORKSHEET
(POSITIVE CONTROLS/TEST COMPOUND)

	Substance Assay	/ed: (	(1) <u>Co</u>	de #91		(	2)				
	(3)	·	(4	)			(5) _	- ,,			
	Date: 18 Nov 8	0		Perf	ormed E	By: <u>Kinc</u>	annon, Si	ummers,	Sauers.	<u>Pull</u> ian	1
	Substance disso	olved	in: (	1)[	MSO		(2)			<del>~~~</del>	
	(3)		(	4)			(5)				
		•	•	# R	evertar	t/Plate	1				
Sub	Conc	98	98A	100	100A	1535	1535A	1537	1537A	1538	1538A
91	0.01 mg/pl	19	21	73	77	9	9	33	4	8	10
91	0.002 mg/pl	20	21	66	69	6	7	4	3	-6	9
91	0.0004 mg/pl	16	23	72	75	9_	7	6	4	6	9
91	0.00008 mg/p1	15	24	36	76	6	10	3	6	6	11
91	0.0000032mg/p1	22	19	34	77	10	9	4	,	9	15
	Spon. Rev.	2	36	76	130	5	6	3	5	2	11
	1								1		

#### Table-5-F SALMONELLA/MICROSOME ASSAY WORKSHEET (POSITIVE CONTROLS/TEST COMPOUND)

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	Substance Assay	)Code #37			(	(2) <u>Code #73A</u>					
	(3) Code #83		(4)	Co	de #53		(5) _	Code	<b>491</b>		
	Date: 4 Dec 8	0		Perf	ormed By	:_Kinc	annon,	Summ <b>e</b> rs,	Sauers,	Pullia	m
	Substance disso	olved in:	(1)		DMSO						
	(3)		_ (4)		······		(5)				
		•	•	# Re	evertant	/Plate	-				
Sub	Conc	98 9	8A	100	100A	1535	1535A	1537	1537A	1538	1538A
37	1.0 mg/pl							Toxic	Toxic		
37	0.2 mg/p1							1	1		
37	0.04 mg/pl							3	3		-t-C-reter-Ton-science
37	0.008 mg/pl							2	3		
37	0.0016 mg/pl							3	3		
37	0.00032 mg/pl		,	,				4	2		
73A	1.0 mg/pl							Toxic	Toxic		
73A	0.2 mg/pl							2	2		
73A	0.04 mg/p1							4	4		
73A	0.008 mg/pl							3	2		
73A 0	0016 mg/p1							2	3		
73A	0.00032 mg/pl							4	3		
83	1.0 mg/pl							3	1		
83	0.2 mg/pl							3	3		
83	<b>0</b> .04 mg/pl							4	3		
83	0.008 mg/p1							4	5		
83	0.0016 mg/pl							5	2		
83	0.00032 mg/p1							5	3		
										: -	~

#### SALMONELLA/ MICROSOME ASSAY WORKSHEET (POSITIVE CONTROLS/TEST COMPOUND) Table-5-F Continuation Page

### # Revertant/Plate

Sub	Conc	98	98A_	100	100A	1535	1535A	1537	1537A	1538	15:::
53	1.0 mg/p1							4	2		_
53	0.02 ing/pl				! <del> </del>				3		
_53_	0.04 mg/p:							4	3		
53	0.003 mg/;1							2	2		
53	0.0016 mg/p1							2	3		
53	0.00032 mg/pl							5	3		
91	0.01 ing/p1							3	2		
91	0.002 mg/p1							3	2		
91	0.0004 mg/pl							4	3		
91	0.00008 mg/pl				-			2	2		
91	0.000016mg/p1							2	2		
91	0.0000032mg/p							2	2		
,.	Spon. Re/							4	6		
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#### Table-5-G SALMONELLA/MICROSOME ASSAY WORKSHEET (POSITIVE CONTROLS/TEST COMPOUND)

	Substance Assa	yed: (	(1) _6	ode #3		(	2)	Code #	73A		
	(3) <u>Code #83</u>		(4	) <u> </u>	ode #53		(5) _	Code #9	1		
	Date: 11 Dec	80	*	_ Perf	ormed B	y: Sauer	rs, Summ	ers, Kir	cannon,	Pulliam	)
	Substance diss	olved	in: (	1)	Ditiso		(2)				
	(3)								-		
	111	,	,		evertan						
Sub	Conc	98	ARP		100A			1537	1537A	1538	15384
37	1.0 mg/p1							3	6	_,	
37	0.2 mg/p1							4	4		
37	0.04 mg/pl							5	9		
37	0.008 mg/pl							4	7		
37	0.0016 mg/pl							7	7		
37	0.00032 mg/pl							4	4		_
73A	1.0 mg/pl							7	6		
73A	0.2 mg/pl							b	5		
73A	0.04 mg/pl							4	6		
73A	0.008 mg/p1							4	9		
73A	0.0016 mg/p1							6	7		
73A	0.00032 mg/p1							4	7		
83	1.0 mg/pl							6	4		
83	0.2 mg/pl							44	6		
83	0.04 mg/p1							4	7		
. 83	0.008 mg/p1							7	_8		
83	0.0016 mg/p1							5	_5		
_83_	0.00032 mg/p1							_1	7		
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#### SAI MONFLLA/ MICROSOME ASSAY WORKSHEET (POSITIVE CONTROLS/TEST COMPOUND) Table-5-G Continuation Page

### # Revertant/Plate

Sub	Lonc	98	98A	100	100A	1535	1535A	1537	1537A	1538	1538A
53	1.0 mg/pl			ļ				6	6		
53_	0.2 mg/p1							5	7		
53	0.04 mg/pl							4	7		
53	1c\pm 800.0				,			4	_10		
53	0.0016 mg/pl							4	7		
53	0.00032 mg/p1							6	6		
									-		
91	0.01 mg/p1							5	7		
91	0.002 mg/p1							6	9		
91	0.0004 mg/p1							5	6		
91	0.00008 mg/p1							7	8		
91	0.000016mg/p1							7	6		
91	0.0000032ng/p							5	7		
-	Spon. Rev.							7	8		
				-							
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#### Table-5-H SALMONELLA/MICROSOME ASSAY WORKSHEET (POSITIVE CONTROLS/TEST COMPOUND)

	Substance Assa	yed:	(1)	ode # 8	33	(	2)			~	
	(3)		(4	.)			(5)				
	Date: 16 Dec	80		_ Perf	ormed B	y: <u>Pull</u>	iam, Summ	ers, Sa	uers, Ke	llner	
					,						
	Substance diss						(2)				
	(3)		(	4)			(5)		<del></del>		
		1	•	# R	evertan	t/Plate	<u>.</u>				
Sub	Conc	98	98A	100	100A	1535	1535A	1537	1537A	1538	1538
83	1.0 mg/p1	9	16						_	12	
83	0.2 mg/pl	$ _{\mathbf{u}}$	17							11	
83	0.04 mg/pl	16	19							14	
83	0.008 mg/pl	14	21							-8	
83	0.0016 mg/p1	17	20							6	
83	0.00032 mg/p1	17	16							6	
	Spon Rev.	25	30							15	
								<del></del>			······································
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#### Table-5-I SALMONEI.LA/MICROSOME ASSAY WORKSHEET (POSITIVE CONTROLS/TEST COMPOUND)

	Substance Assa	yed:	1: (1) <u>Code #53</u> (2)								
	(3)		(4				(5) _	٠,	<del></del>		
	Date: 16 Dec	80		_ Perf	ormed B	y: <u>Pull</u> :	iam. Kel	lner. Sa	uers. S	ummers	
	Substance diss	olved	in: (	1) <u>DM</u>	50		(2)				
	(3)		(	4)		<del></del>	(5)		·		
		,	•	# R	levertan	t/Plate					
Sub	Conc	98	98A	100	100A	1535	1535A	1537	1537A	1538	1538/
53	1.0 mg/p1	14	18							9	
53	0.2 mg/p1	16	17							14	
53	0.04 mg/p1	19	17							_10	
53	0.008 mg/p1	15	10							77	
53	0.0016 mg/rl	17	20							9	
53	0.00032 mg/pl	11	8							7	
-											
	Spon. Rev.	25	30							15	
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#### Table-5-J SALMONELLA/MICROSOME ASSAY WORKSHEET (POSITIVE CONTROLS/TEST COMPOUND)

	Substance Assay	/ed: (	(1) <u>Co</u>	de #91		(	2)				
	(3)		(4	)			(5)	<del></del> -			
	Date: 16 Dec	80		_ Perf	ormed B	y: <u>Pull</u>	iam. Sauc	rs. Sum	mers. Ke	llner	
	Substance disso	olved	in: (	1) <u>DMS</u>	50	···	(2)				
	(3)		(	4)			(5)		<del></del>		
		1	•	# R	evertan	t/Plate	<u>.</u>				
Sub	Conc	98	98A	100	100A	1535	1535A	1537	1537A	1538	1538A
91	0.01 mg/p1	8	19							6	 
91	0.002 mg/p1	14	22							10	
91	0.0004 mg/p1	11	18							_12	
91	0.00008 mg/pl	13	19							10	
91	0.000016mg/p1	14	23							14	
91	0.0000032mg/pl	13	14	<u> </u>						3	
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	Spon. Rev.	25	30							15	
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Table-6-A

#### Salmonella/Microsome Assay

DMSO

Subs	tai	nce As	ssayed:	Code	#37			Dissolv	ed In:	DMSO		
Date	e: .	12 No	08 v	Per	formed	By: _	Pulliam	, Sauers				
Test Compound and Concentration		TA (a)	(b)	E act	(c)	C	E-C (d)	E-C act	CAV (e)	C _{AV} act	HUTAR (f)	MUTA act
1.0 mg/pl	TA	98	12	13	22	26						
0.2 mg/pl	TA	98	12	19	22	26						
0.04 mg/pl	TA	98	14	28	22	26			**			
0.008 mg/pl	TA	98	21	23	22	26						
0.0016 mg/p1	TA	98	18	22	22	26						
0.00032 mg/pl	TA	98	18	19	22	26			<del></del>			
1.0 mg/p1	TA	100	60	66	115	112						
0.2 mg/pl	TA	100	83	92	115	112						
0.04 mg/p1	TA	100	89	97	115	112						
0.003 mg/p1	TA	100	75	88	115	112						
0.0016 mg/pl	TA	100	84	77	115	112						
0.00032 mg/p1	TA	100	112	89	115	112						
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(a)= tester strain: (b)=no. of experimental revertant colony forming units. (c)=no. of assayed spontaneous revertants: (d)=no. revertants in excess of the assayed spontaneous revertant rate: (e)=spontaneous reversion rate calculated from historical data: (f) =  $E-C/C_{AV}$ : act = activation with S-9

Table-6-A

#### Salmonella/Microsome Assay

Sub	stance As	sayed:	Cod	e #37			_ Dissolv	ed In: _	DMSO		
Dat	e: <u>12 N</u>	ov 80	Per	formed	By: _	Pulliar	n, Sauers				
Test Compound and Concentration	(a)	E (b)	E act	(c)	C	E-C (d)	E-C act	C _A y (e)	C _{Ay} act	HUTAR (f)	MUTA- act
1.0 mg/pl	TA 1535	6	5	13	5						
0.2 mg/pl	TA 1535	10	4	13	5						
0.0004 mg/pl	TA 1535	6	7	13	5		2	*	13.3		0.15
0.008 mg/p1	TA 1535	11	6	13	5		1		13.3		0.8
0.0016 mg/p1	TA 1535	10	12	13	5		7		13.3		0.53
0.00032 mg/p1	TA 1535	12	6	13	5		1		13.3		0.8
1.0 mg/pl 0.2 mg/pl	TA 1537	6	7	11	10						
0.04 mg/pl	TA 1537	9	110	11	10		100		7.5		13.3
0.008 mg/pl	TA 1537	8	8	11	10				ļ		
0.0016 mg/pl	TA 1537	7	12	11	10		2		7.5		0.27
0.00032 mg/p1	TA 1537	6	7	11	10	<u> </u>	ļ		ļ		
	<del> </del>	ļ	ļ			ļ			<u> </u>		 
	<u> </u>	<u> </u>	ļ	ļ	ļ		<u> </u>	ļ			
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(a)=tester strain: (b)= no. of experimental revertant colony forming units: (c)=no of assayed spontaneous revertants: (d)= no. revertants in excess of the assayed spontaneous revertant rate: (e)=spontaneous reversion rate calculated from historical data: (f) =  $E-C/C_{AV}$ : act = activation with S-9

Table-6-A

#### Salmonella/Microsome Assay

Substa	nce Assayed:_	Code #37		Dissolved	In:	DMS0
Date:	12 Nov 80	Performed By	: Kincannon	. Sauers	Summe	rs. Pulliam

Test Compound and Concentration	(a)	(b)	E act	(c)	C act	E-C (d)	E-C act	CAV (e)	C _{AV} act	MUTAR (f)	MUTA act
1.0 mg/pl	TA 1533	5	7	10	14			w			
0.2 mg/p1	TA 1538	10	21	10	14		7		17.1		0.41
0.04 mg/p1	TA 1538	10	25	10	14		11		17.1		0.64
0.008 mg/pl	TA 1538	9	17	10	14		3		17,1		0.17
0.0016 mg/p1	TA 1538	12	14	10	14	2		8.3		0,24	
0.00032 mg/p1	TA 1538	5	13	10	14			 	<u></u>		
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⁽a)- tester strain: (b)=no. of experimental revertant colony forming units: (c)=no. of assayed spontaneous revertants: (d)=no. revertants in excess of the assayed spontaneous revertant rate: (e)=spontaneous reversion rate calculated from historical data: (f) - E-C/ $C_{AV}$  act = activation with S-9

Table-6-B

# MUTAGENIC ACTIVITY RATIO WORKSHEET Salmonella/Microsome Assay

# Substance Assayed: Code #73A Dissolved In: DMSO Date: 12 Nov 80 Performed By: Pulliam, Sauers

Test Compound and Concentration	TA (a)	(b)	E act	(c)	C act	(d)	E-C act	CAV (e)	C _A y act		37.
1.0 mg/pl	TA 98	9	14	22	26						
0.2 mg/p1	TA 98	14	21	22	26				<del></del>		
0.04 mg/pl	TA 98	16	21	22	26				·	:	
0.008 mg/pl	TA 98	14	16	22	26						<b>-</b> -
0.0016 mg/pl	TA 98	12	24	22	26	····· 114 41 <del>44 44</del>					with the ray
0.00032 mg/pl	TA 98	13	15	22	26						
				<u> </u>							_
1.0 mg/pl	TA 100	47	54	115	112					-	
0.2 mg/p1	TA 100	63	68	115	112						<b></b> -
0.04 mg/pl	TA 100	80	90	115	112					<u> </u>	-
0,008 mg/p1	TA 100	65	90	115	112				·	;	
0,0016 mg/pl	TA 100	74	95_	115	112					:	_
0.00032 mg/p1	TA 100	76	80_	115	112					,	
		<u> </u>							-	i	
	 -									,	
		<u> </u>								i .	
	1	1								1	

⁽a)= tester strain: (b)=no. of experimental revertant colony forming units: (c)-no. of assayed spontaneous revertants: (d)=no. revertants in excess of the assayed spontaneous revertant rate: (e)=spontaneous reversion rate calculated from nustorical data. (f) =  $E-C/C_{AV}$ : act = activation with >-9

Table-6-B

#### Salmonella/Microsome Assay

Substance Assayed: Code #73A Dissolved In: DMSO

Date	e: <u>12 No</u>	<u>08 v</u>	Per	formed	Ву: _	Pullia	m, Sauer	5	. ———. —	
Test Compound and Concentration	TA (a)	(b)	E act	(c)	Cact	E-C (d)	E-C act	CAy (e)	C _A v act	MUTAR MUTA (f) act
1.0 mg/pl	TA 1535	9	5	13	5					,
0.2 mg/pl	TA 1535	8	7	13	5		2		13.3	0.15
0.04 mg/p1	TA 1535	10	7	13	5		2		13.3	0.15
Q.008 mg/pl	TA 1535	7	8	_13	5		3		13.3	0.45
0.0016 mg/p1	TA 1535	7	8	13	5		3		13.3	1 0.45
0.00032 mg/p1	TA 1535	5	9	13_	5		4		13.3	0.61
1.0 mg/pl	TA 1537	4	2	11	10					,
0.2 mg/pl	TA 1537	5	6	11_	10					
0.04 mg/pl	TA 1537	8	6	1.11.	10					<u>'</u>
0.008 mg/p)	TA 1537	10	6	11_	10					
0.0016 mg/pl	TA 1537	7	9	<u></u>	10					
0.00032 mg/p)	TA 1537	_5	7	11_	10_					
	<del> </del>								 	
										1
1.0 mg/p]  0.2 mg/p]  0.34 mg/p]  0.008 mg/p]  0.0016 mg/p]	TA 1537 TA 1537 TA 1537 TA 1537 TA 1537	4 5 8 10 7	2 6 6 6	11 11 11 11	10 10 10 10		4		13.3	

(a)- tester strain: (b)=no. of experimental revertant colony forming units: (c)=no. of as ayed spontaneous revertants: (d)=no, revertants in excess of the assazed spontaneous revertant rate: (e)=spontaneous reversion rate calculated from historical data (f) = E-C/C_{AV}: act = activation with S-9

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Table-6-B

Substance Assayed: Code #73A

#### MUTAGENIC ACTIVITY RATIO WORKSHEET

#### Salmonella/Microsome Assay

Dissolved In: DMSO

Dat	e: <u>12 Nov</u>	80	Per	formed	Ву: _	Sauer	<u>s</u>				
Test Compound and Concentration	(a)	E (b)	E act	(c)	Cact	E-C (d)	E-C act	CAV (e)	C _{AV} act	HUTAR (f)	MU-, act
1.0 mg/pl	TA 1538	8	15	10	14		1		17.1		0.06
0.2 mg/pl	TA 1538	10	14	10	14						
0.04 mg/pl	TA 1538	8	22	10	14		6	<del></del>	17.1		0.35
0.008 mg/pl	TA 1538	6	12	10	14						
0.0016 mg/pl	TA 1538	10	14	10	14			-			
0.00032 mg/p1	TA 1538	8	14	10	14						
							-				

(a)= tester strain: (b)=no, of experimental revertant colony forming units: (c)=no, of assayed spontaneous revertants: (d)=no, revertants in excess of the assayed spontaneous revertant rate: (e)=spontaneous reversion rate calculated from nistorical data: (f) =  $E-C/C_{AV}$ : act = activation with S-3

Table-6-C MUTAGENIC ACTIVITY RATIO WORKSHEET

Subs	tance As:	sayed:	Cod	e #83			Dissolv	ved In: _	DMS	)	
Date	: 18 N	ov 80	Per	formed	By:	Pulliam,	Sauers	····			
-							-				-
Test Compound and Concentration	(a)	(b)	E act	(c)	act	E-C (d)	E-C act	CAV (e)	C _{AV} act	HUTAR: (f)	MUTA
1.0 mg/p]	TA 98	11_	10	2	36	9		23.1		0.39	
0.2 mg/pl	TA 98	19_	22	2	36	17.		23.1		0.74	
0.04 mg/pl	TA 8	_17_	19	2	36	15	-	23.1		0.65	
	TA SA	. 19	22	2	36	17		23.1		0.74	<del></del>
0.0016_mg/p1	TA 98	20	17	2	36	18		23.1		0.78	
0.00032 mg/pl	TA 98	_12_	15	2	36	_10		23.1		0.43	
										<u> </u>	
	TA 100	47	40		1.30			100		0.05	-
0.2 mg/pl	TA 100	_81			130	5		106		0.05	
1	TA 100	84	96		130	8		196		0.07	
0.008_mg/pl	TA_100_	_85	.84	76	130	9		196		0.98	
0.0016_mg/pl	TA_100	66	60	76	130						
0.00032_mg/pl	TA_:00_	5)	83	76	30						
Salar St. Str. Market St. Strate St. 1975, again											

(a)= tester strain: (b)=no, of experimental revertant colony forming units: (c)=no, of assayed spontaneous revertants: (d)=no, revertants in excess of the assayed spontaneous revertant rate: (e)=spontaneous reversion rate calculated from historical data: (f) -  $E=C_{V}C_{AV}$ : act - activation with S-9

Table-6-C

#### Salmonella/Microsome Assay

Substance Assayed: Code #83 Dissolved In: DMSO

Date	e: <u>18 No</u>	08 vo	Per	formed	Ву: _	Pulliam,	Sauers	<del></del>		**************************************	
Test Compound and Concentration	TA (a)	(b)	E act	(c)	C act	E-C (d)	E-C act	CAV (e)	CAV	MUTAR (f)	MUT: act
1.0 mg/p1	TA 1535	4	6	5	6						
0.2 mg/pl	Ta 1535	9	7	5	6	4	1	9.4	13.3	0.43	0.03
0.04 mg/pl	TA 1535	5	7	5	6				13.3		0.08
0.008 mg/p1	TA 1535	6	6	5	6	J		9.4		0.11	
0.0016 mg/p1	TA 1535	6	4	5	6	1		9.4		0.11	
0.00032 mg/p1	TA 1535	6	7	5	6	1	1	9.4	13.3	0.11	0.08
1.0 mg/pl	TA 1537	1	1	3	5						
0.2 mg/pl	TA 1537	3	4	3	5				ļ		
0.04 mg/p1	TA 1537	5	3	3	5	2		6.1	ļ	0.33	
0.008 mg/pl	TA 1537	3	3	3	5			·			
0.0016 mg/p1	TA 1537	1 1	4	3	5						
0.00032 mg/pl	TA 1537	3	2	3	5						
								`			

(a)= tester strain: (b)=no, of experimental revertant colony forming units: (c)=no, of assayed spontaneous revertants: (d)=no, revertants in excess of the assayed spontaneous revertant rate: (e)=spontaneous reversion rate calculated from hi torical data. (f) =  $E-C/C_{AV}$ : act = activation with 5-9

Table 6-C

Substance Assayed: Code #83

#### MUTAGENIC ACTIVITY RATIO WORKSHEET

#### Salmonella/Microsome Assay

___ Dissolved In: __DMSO

Date	: 18 1	lov 80	Per	formed	Ву: _	Sauer	<u>`s</u>				
Test Compound and Concentration	†A (a)	E (b)	E act	(c)	Cact	E-C (d)	E-C act	CAV (e)	CAV	HUTAR (f)	MUTA act
1.0 mg/pl	TA 1538	4	7	2	11	2		8.3		0.24	
0.2 mg/pl	TA 1538	3	12	2	11	١	1	8.3	17.1	0.12	0.06
0.0% mg/pl	TA 1538	G	12	2	11	4	1	8.3	17.1	0,48	0.06
0.008 mg/p1	TA 1538	7	15	2	11	5	4	8.3	17.1	0.61	0.23
0.0016 mg/p1	TA 1538	13	7	2	11	11		8.3		1.32	
0.00032 mg/p	1 TA 153	8 6	11	2	11	4		8.3		0.48	
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	1										
	1	1	1		1				1	1	1

(a)= tester strain. (b)=no. of experimental revertant colony forming units: (c)=no. of assayed spontuneous revertants: (d)=no. revertants in excess of the assayed spontaneous revertant rate: (e)=spontaneous reversion rate calculated from historical data: (f) = E-C,  $C_{AV}$ : act = activation with S-9

Table-6-D

#### Salmonella/Microsome Assay

Substance Assayed:	Code #53	Dissolved In	1: DMSO
Date: 18 Nov 80	Performed By: Pu	lliam, Sauers	

Test Compound and Concentration	TA (a)	(b)	E act	(c)	C act	E-C (d)	E-C act	CAV (e)	C _A y act	HUTAR (f)	ATUM act
1.0 mg/pl	TA 98	17	25	2	36	15		23.1	· · · · · · · · · · · · · · · · · · ·	0.65	
0.2 mg/pl	TA 98	15	20	2	36	13		23.1		0.56	
0.04 mg/pl	TA 98	17	18	2	36	15		23.1		0.65	
0.008 mg/pl	TA 98	17	23	2	36	15		23.1		0.65	
0.0016 mg/pl	TA 98	16	25	2	36	14		23.1		0.60	
0.00032 mg/pl	TA 98	13	25	2	36	11		23.1		0.48	<del></del>
1.0 mg/pl	TA 100	66	74	76	130						
0.2 mg/pl	TA 100	89	86	76	130	13		106		0.12	
0.04 mg/pl	TA 100	75	85	76	130						
0.008 mg/pl	TA 100	84	93	76	130	88		106		0.08	
0.0016 mg/pl	TA 100	87	93	76	130	11		106		0.10	
0.00032 mg/pl	TA 100	70	90	76	130						
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(a)= tester strain: (b)=no. of experimental revertant colony forming units: (c)=no. of assayed spontaneous revertants: (d)=no. revertants in excess of the assayed spontaneous revertant rate: (e)=spontaneous reversion rate calculated from historical data: (f) = E-C/C_{AV}: act = activation with S-9

Table-6-D MUTAGENIC ACTIVITY RATIO WORKSHEET

Substance Assayed: Code #53 Dissolved In: DMSO

Date	): -	18 No	v 80	Per	formed	Ву: _	<u>Pulliam,</u>	Sauers	<u></u>			
Test Compound and Concentration		`A (a)	(p)	E act	(c)	C act	E-C (d)	E-C act	CAV (e)	C _A y act	MUTAR (f)	MUTA act
1.0 mg/pl	TA	1535	6	4	5	6			9,4	13.3	0.11	
0.2 mg/pl	TA	1535	8	7	5	6	3	1	9.4	13,3	0.32	0.08
0.04 mg/p]	TA	1535	9	9	5	6	4	3	9.4	13.3	0.43	0.23
1a\pm_800.0	TA	`535	6	9	5	ű	7	7	9.4	13.3	0.76	0.08
0.0016 mg/p1	TA	1535	6	9	5	6	1	3	9.4	13.3	0.11	0.23
0,00032 mg/p1	TA	1535	13	8	5	6	8	2	9,4	13.3	0.89	0.15

TA 1537

TA 1537

TA 1537

1.0 mg/pl

 $0.2 \, \text{mg/pl}$ 

0.04 mg/pl

0.008 mg/pl	TA 1537	6	5	3	_5	3		6.1		0.49	
0.0016 Jng/pl	TA 1537	9	6	3	5	6		6.1	7.5	0.98	0.13
	ì	١,	6	3	5		1		7.5		0.13
											<del> </del>
	1										
	0.0016 mg/pl	0.0016 mg/pl TA 1537	0.008 mg/pl TA 1537 6 0.0016 Jng/pl TA 1537 9 0.00032 mg/pl TA 1537 3	0.0016 mg/pl TA 1537 9 6	0.0016 mg/pl TA 1537 9 6 3	0.0016 mg/p1 TA 1537 9 6 3 5	0.0016 mg/pl TA 1537 9 6 3 5 6	0.0016 mg/pl TA 1537 9 6 3 5 6 1	0.0016 mg/p1 TA 1537 9 6 3 5 6 1 6.1	0.0016 mg/p1 TA 1537 9 6 3 5 6 1 6.1 7.5	0.0016 mg/p1 TA 1537 9 6 3 5 6 1 6.1 7.5 0.98

6.1

0.16

(a)= tester strain: (b)=no. of experimental revertant colony forming units: (c)=no. of assayed spontaneous revertants: (d)=no. revertants in excess of the assayed spontaneous revertant rate: (e)=spontaneous reversion rate calculated from historical data: (f) =  $E-C/C_{AV}$  act = activation with S-9

Table-6-D MUTAGENIC ACTIVITY RATIO WORKSHEET

Sub	stance As	sayeo:	<u> </u>	ode #5	3		_ D12201/	/ea in: -	บกรบ		
Date	e: <u>18 No</u>	08 v	Per	formed	Ву: _	Pu] ] 1	iam, Saue	ers		-	
Test Compound and Concentration	(a)	(b)	E act	(c)	Cact	E-C (d)	E-C act	C _{AV} (e)	C _{AV} act	HUTAR (f)	MUT: act
1.0 mg/pl	TA 1538	14	13	2	11	12	2	8.3	17.1	1.45	0.13
0.2 mg/pl	TA 1538	9	9	2	11	7		8.3		0.84	<u> </u>
0.04 mg/p1	TA 1538	5	11	2	11	3		8.3		0.36	
0.008 mg/pl	TA 1538	111	16	2	11	9	5	8.3	17.1	1.08	0.24
0.0016 mg/pl	TA 1538	6	16	2	11	4	5	8.3	17.1	0.48	0.29
0.00032 mg/p1	TA 1538	9	11	2	11	7	ļ	8.3	ļ	0.84	
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(a)= tester strain: (b)=no. of experimental revertant colony forming units: (c)=no. of assayed spontaneous revertants: (d)=no, revertants in excess of the assayed spontaneous revertant rate: (e)=spontaneous reversion rate calculated from historical data: (f) =  $E-C/C_{AV}$ : act = activation with S-9

Table 6-E
MUTAGENIC ACTIVITY RATIO WORKSHEET

Substance Assayed: <u>Code #91</u>	Dissolved In: DMSO
Date: 18 Nov. 80 Performed By:	Pulliam, Sauers

Test Compound and Concentration	Ţλ (a)	£ (b)	E act	(c)	C act	(d)	E-C act	CAV (e)	C _{AV} act	MUTAR:	MUTA act
0.01 mg/plate	TA 98	19	21	2_	36	17		23.1		0.74	
2 x 10 ⁻³	TA 98	20	21_	2_	36	18		23,1		0.78	
4 x 10 ⁻⁴	TA 98	12_	23	_2_	36	10		23,1	~~~·	0,43	
8 x 10 ⁻⁵	TA 98	_15	24	_2_	36_	13		23,1		0,56	
3.2 × 10 ⁻⁶	TA 98	_22	19	_2_	36	20		23,1		0.87	<del></del>
0.01 mg/plate	TA 100	73	.77	76	130						
2 x 10 ⁻³	TA_100	66	69	76_	130						
6 × 10-4	TA_100		. 75	76.	130						
8 x 10-5	TA 100	36	76	76	130						,
3.2 x 10 ⁻⁶	TA 100	84_	_77	76	130						
0.01 mg/plate	TA 1535	9	9	5	. 6.	4	3	9.4	13.3	0.43	0.2
2 x 10 ⁻³	TA 1535	6_	7	5	6 _		1_	9.4	13.3.	0.11	0.0
4 x 10 ⁻⁴	TA 1535	9	7	5	6	4	11	_9.4	13.3	0.43	_0.0
8 x 10 ⁻⁵	   <u>TA_ 1535</u>	6	10_	5_	6	1	4_	9.4	13.3	0.11	_0.3
3.2 × 10 ⁻⁶	TA 1535	_10.	9	5_	6_	5	3	9.4.	13.3	0.53	2.2

⁽a)= tester strain: (b)=no, of experimental revertant colony forming units: (c)=no, of assayed spontaneous revertants: (d)=no, revertants in excess of the assayed spontaneous revertant rate: (e)=spontaneous reversion rate calculated from historical data: (f) =  $E-C/C_{AV}$  act = activation with S-9

TAble 6-E
MUTAGENIC ACTIVITY RATIO WORKSHEET

Substance Assayed:	Code # 91	Dissolved I	in: <u>DMSO</u>
Date: <u>18 Nov 80</u>	Performed By:	Pulliam, Sauers	

Test Compound	TA (a)	(b)	E	(c)	C	E-C (d)	E-C act	CAV (e)	CAV	HUTAR (f)	MUT -
Concentration	(4)				400	(0)		(6)		\','	!
0.01 mg/plate	TA 1537	3	4	3	5						·
2 x 10 ⁻³	TA 1537	4	3	3	5	1		6.1		0.16	!
4 × 10 ⁻⁴	TA 1537	6	4	3	5	3		6.1		0,49	ì
8 x 10 ⁻⁵	TA 1537	3	6_	3	5		111		7.5		C.13
3.2 × 10-6	ግለ 1537	4	7	3	5	11	2	6.1	7.5	0,16	1.2.
											İ
0.01 mg/plate	TA 1538	8	10	2	_11_	.6		8.3		0.73	
2 × 10 ⁻³	TA 1538	6	8	2_	11	4		8.3		0.48	!
4 × 10 ⁻⁴	T.\ 1530	6	9	2	11	4		8.3		0.48	1
8 x 10 ⁻⁵	TA 1538	6	11	2	11	4		8.3		0,48	1
3.2 x 10 ⁻⁶	TA 1538	9	15	2	11	7	4	8.3	17.1	0.84	0.23
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⁽a)= tester strain: (b)=no. of experimental revertant colony forming units: (c)=nc. of assayed spontaneous revertants: (d)=no. revertants in excess of the assayed spontaneous revertant rate: (e)=spontaneous reversion rate calculated from historical data: (f) =  $E-C/C_{AV}$ : act = activation with S-9

Table-6-F
MUTAGENIC ACTIVITY RATIO WORKSHEET
Salmonella/Microsome Assay

Subs	stance As	sayed:	Cod	e # 37			Dissolv	ed In: j	DMSO		
Date	e: <u>11 De</u>	c 80	Per	formed	Ву: _	Pulli	am. Saue	rs	·		
Test Compound and Concentration	(a)	E (b)	E act	(c)	C ac+	E-C (d)	E-C act	CAV (e)	C _{AV} act	HUTAR (f)	MUT/ ac
1.0 mg/p1	TA 1537	3	6	7	8			- <del> </del>			
0.2 mg/p1	TA 1537	4	4	7	8						<u> </u>
0.04 mg/pl	TA 1537	5	9	7	8		11		7.5		0.13
0.008 mg/pl	TA 1537	4	7	7	8						
0.0016 mg/pl	TA 1537	7	7	7	8				<u></u>		
0.90032 mg/pl	TA 1537	4	4	7	8						
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											-

(a)= tester strain: (b)=no. of experimental revertant colony forming units: (c)=no. of assayed spontaneous revertants: (d)=no. revertants in excess of the assayed spontaneous revertant rate (e)=spontaneous reversion rate calculated from historical data: (f) =  $E-C/C_{AV}$ : act = activation with S-9

Table-6-G

Substance Assayed: Code #53

#### MUTAGENIC ACTIVITY RATIO WORKSHEET

#### Salmonella/Microsome Assay

__ Dissolved In: <u>DMSO</u>

Date	e: <u>11 De</u>	c 80	Per	formed	By: _	Pullia	ım, Sauer	's	<del></del>		
Test Compound and Concentration	(a)	E (b)	E act	(c)	C	E-C (d)	E-C act	CAV (e)	C _{AV} act	HUTAR (f)	MUT;
	TA 1537	6	6	7	8						
0.2 mg/pl	TA 1537	5	7	7	8						
0.04 mg/pl	TA 1537	4	7	7	8				<u> </u>		
0.008 mg/pl	TA 1537	4	10	7	8		2	L	7.5		0.20
0.0016 mg/pl	TA 1537	4	7	7	8						
0.00032 mg/pl	TA 1537	6	6	7	8						
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(a)= tester strain: (b)=no. of experimental revertant colony forming units: (c)=no. of assayed spontaneous revertants: (d)=no. revertants in excess of the assayed spontaneous revertant rate: (e)=spontaneous reversion rate calculated from historical data. (f) =  $E-C/C_{AV}$ : act = activation with S-9

Table-6-H

Substance Assayed: Code #83

#### MUTAGENIC ACTIVITY RATIO WORKSHEET

#### Salmonella/Microsome Assay

Dissolved In: __DMSO

Date	e: <u>11 De</u>	c 80	Per	formed	By: _	Pulliam	, Sauers	<del></del>			
Test Compound and Concentration	(a)	(b)	E act	(c)	Cact	E-C (d)	E-C act	CAV (e)	C _A y act	MUTAR (f)	MUTA act
1.0 mg/pl	TA 1537	6	4	7.3	8						·
0.2 mg/pl	TA 1537	4	6	7.3	3						
0.04 mg/pl	TA 1537	4	7	7.3	8						
0.008 mg/pl	TA 1537	7	8	7.3	8			· · · · · · · · · · · · · · · · · · ·			
0.0016 mg/pl	TA 1537	5	5	7.3	8						
0.00032 mg/pl	TA 1537	7	7	7.3	8						
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(a)= tester strain: (b)=no. of experimental revertant colony forming units: (c)=no. of assayed spontaneous revertants: (d)=no. revertants in excess of the assayed spontaneous revertant rate: (e)=spontaneous reversion rate calculated from historical data: (f) =  $E-C/C_{AV}$ : act = activation with S-9

## Table 6-I MUTAGENIC ACTIVITY RATIO WORKSHEET

#### Salmonella/Microsome Assay

Subs	stance As	sayed:	Co	de #91			Dissolv	ed In:	DMSO		
Date	:: <u> </u>	ec 80	Per	formed	Ву: _	Sauers.	Fulliam		<del>-</del>		
Test Compound and Concentration	(a)	E (b)	E act	(c)	Cact	E-C (d)	E-C act	CAY (e)	CAV	HUTAR (f)	MUTAI act
0.01 mg/plat	TA 153	5_		7	8			· · · · · · · · · · · · · · · · · · ·			
2 x 10-3	TA 153	6_	9	7	8		1		7.5		0.13.
4 × 10-4	TA 153	5_	6_		8_						<u> </u>
8 x 10-5	TA 1537	. 7	8_		8						
1.6 × 10-5	TA 1537	7	6_		8						
3.2 x 10 ⁻⁶	TA 1537	5	7		8_						
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(a)= tester strain: (b)=no. of experimental revertant colony forming units: (c)=no. of assayed spontaneous revertants: (d)=no. revertants in excess of the assayed spontaneous revertant rate: (e)=spontaneous reversion rate calculated from historical data: (f) =  $E-C/C_{AV}$ : act = activation with S-9

Table-6-J

#### Salmonella/Microsome Assay

Subs	stance As	sayed:	Cod	e #73A		· · · · · · · · · · · · · · · · · · ·	_ Dissolv	ed In: _	DMSO		
Date	: <u>11 De</u>	c 80	Per	formed	Ву: _	Pullia	am. Sauer	'S			
Test Compound and Concentration	(a)	E (b)	E act	(c)	C act	E-C (d)	E-C act	CAV .!	C _{AV} act	MUTAR (f)	MUTAR
1.0 mg/pl	TA 1537	7	6	7.3	8						
0.2 mg/pl	TA 1537	6	5	7.3	8						
0.04 mg/pl	TA 1537	4	6	7.3	8						<b></b>
Q.QQ8_mg/pl	TA 1537	4	9	7.3	8		1		7.5		0.13
0.0016 mg/pl	TA 1537	6	7	7.3	8						
0.00032 mg/p1	TA 1537	4	7	7.3	8						
								<del> </del>			<del></del>
	-										
	<del>                                     </del>	<del> </del>	<del> </del>	<del> </del>	<del> </del> -	<del> </del>	<u> </u>		<del> </del>	<del> </del>	

(a)= tester strain: (b)=no. of experimental revertant colony forming units: (c)=no of assayed spontaneous revertants: (d)=no. revertants in excess of the assayed spontaneous revertant rate: (e)=spontaneous reversion rate calculated from historical data: (f) = E-C/ $\cup$ AV: act = activation with S-9

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#### Table-6-K

#### MUTAGENIC ACTIVITY RATIO WORKSHEET

#### Salmonella/Microsome Assay

Substa	ince Assayed:	Code #53	Dissolved In: DMSO
Date:	16 Dec 80	Performed By:	Pulliam, Sauers

Test Compound and Concentration	TA (a)	(p)	E act	(c)	C act	E-C (d)	E-C act	C _A y (e)	CAV	fiutar (f)	MUTAR
1.0 mg/pl	TA 98	14	18	25	30						
0.2 mg/pl	TA 98	16	17	25	30				· · · · · · · · · · · · · · · · · · ·		
0.04 mg/pl	TA 98	19	17	25	30						
0.008 mg/pl	TA 98	15	10	25	30						
0.0016 mg/pl	TA 98	17	20	25	30						
0.00032 mg/pl	TA 98	11	8	25	30						
1.0 mg/p1	TA 1538	9		15	18						
0.2 mg/pl	TA 1538	14		15	18						
0.04 mg/pl	TA 1538	10		15	18						
0.008 mg/pl	TA 1538	7_		15	18						
0.0016 mg/p1	TA 1538	9	! !	15	18						
0.00032 mg/pl	TA 1538	7		15	18						
<u></u>											
	l <del>L</del>										
							1				

⁽a)= tester strain: (b)=no. of experimental revertant colony forming units: (c)=no. of assayed spontaneous revertants: (d)=no. revertants in excess of the assayed spontaneous revertant rate: (e)=spontaneous reversion rate calculated from historical data: (f) =  $E-C/C_{AV}$ : act = activation with S-9

Table-6-L

#### Salmonella/Microsome Assay

Substa	ance	Assayed:_	Code #83			Dissolved	In:	DMSO
Date:	16	Dec 80	Performed	By:	Pulliam,	Sauers		

Test Compound and Concentration	TA (a)	(b)	E act	(c)	Cact	E-C (d)	E-C act	CAY (e)	C _A y act	MUTAR (f)	MUTAR act
1.0 mg/pl	TA 98	9	16	25	30						
0.2 mg/pl	TA 93	11	17	25	30						
0.04 mg/pl	TA 98	16	19	25	30						
0.008 mg/pl	TA 98	14	21	25	30						
0.0016 mg/pl	TA 98	17	20	25	30						<b></b>
0.00032 mg/pl	TA 98	17	16	25	30				<del></del>		
1.0 mg/pl	TA 1538	10		15	18						
0.2 mg/pl	TA 1538	11		15	18						
0.04 mg/pl	TA 1538	14		15	18						
0.008 mg/p1	TA 1538	8		15	18						
0.00032 mg/pl	TA 1538	6		15	18	·					
											<u> </u> 
	! 	<del> </del>									
	<u> </u>			<u> </u>		·					

(a)= tester frain: (b)=no. of experimental revertant colony forming units: (c)=no. of assayed spontaneous revertants: (d)=no. revertants in excess of the assayed spontaneous revertant rate: (e)=spontaneous reversion rate calculated from historical data: (f) =  $E-C/C_{AV}$ : act = activation with S-9

Table-6-M

#### Salmonella/Microsome Assay

Subs	Code #91				_ Dissolv	ed In:	DMSO				
Date	: <u>16 De</u>	ec 80	Per	formed	By: _	Pulliam	, Sauers				
Test Compound and Concentration	TA (a)	(b)	ë act	(c)	C act	E-C (d)	E-C act	CAV (e)	Сду	HUTAR (f)	MUT: act
0.001	TA 98	8_	19	25	30						
0.002 mg/ml	TA 98	14	22	25	30						
0.0004 mg/pl	TA 98	11_	18	25	30						
0.00008mg/pl	TA 98	13	19	25	30						
0.000016mg/p1	TA 98	14	23	25	30						
0.0000032mg/pl	TA 98	13	14	25	30						
D.01 mg/pl	TA 1538	6		15	18						
0.002 mg/p1	TA 1538	10		15	18						
0.0004 mg/pl	TA 1538	12		15	18				<u> </u>		
0.00008 mg/pl	TA 1538	10	<u> </u>	15	18						
1 1 1 1 1 2 1 2 1 1 1 1 1 1 1 1 1 1 1 1	TA 1538	14		15	19	 					
D.0000032mg/pl	TA 1538	8	<u></u>	15	18				<u> </u>		
	! <b>!</b>		<u></u>							<u> </u>	<del> </del>
	l L										

(a)= tester strain: (b)=no. of experimental revertant colony forming units: (c)=no. of assayed spontaneous revertants: (d)=no. revertants in excess of the assayed spontaneous revertant rate: (e)=spontaneous reversion rate calculated from historical data (f)  $E-C/C_{\rm AV}$ : act = activation with S-9

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